IMMUNOLIPOSOME ASSAY-METHODS AND PRODUCTS

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with support of the Government of the United States of America by virtue of Grant Numbers CA 24553 and CA 00718, awarded by the National Institutes of Health. The Government has certain rights in this invention.

FIELD OF THE INVENTION

This invention is directed to a homogeneous solid-state immunoliposome assay and to products useful therein, especially in kit form. The assay utilizes the lateral phase separation of an antigenic liposome resulting in the destabilization and lysis of the liposome which may be quantified and employed in determining the presence and/or concentration of antigens, antibodies and like agents in biological fluids.

BACKGROUND OF THE INVENTION

High volume screening assays are commonly employed for detecting the presence of, and quantitatively measuring antigenic materials, antibodies and analytes in biological samples. For example, radioimmunoassay (RIA) techniques are commonly employed for clinical diagnostics. However, RIA procedures are often incompatible with large scale screening programs. Radiotracers, by their very nature, are of limited stability and they require special handling during use, special disposal techniques and sophisticated instrumentation.

Other immunoassay methods currently available include fluorescent and enzymatic techiques. Generally, these assays require a separation step, either by filtration or centrifugation in order to be interpreted. These separation requirements make the assay methods slow and difficult to automate.

Liposomes have previously been reported as useful components for immunoassays. For example, McConnell et al., U.S. Pat. No. 3,887,698, describe the use of liposomes containing stable free radicals in an electron paramagnetic resonance (EPR) monitored immunoassay. Mandle et al., U.S. Pat. No. 4,372,745, describe the use of liposomes as fluorescer containing microcapsules, useful in an immunoassay. This assay requires the use of a detergent such as, Triton X-100 to break the liposomes and release the fluorescent compound. Liposomes have also been employed as a marker carrier in an immunoassay described by Ullman et al., U.S. Pat. No. 4,193,983. Markers used in this assay included fluorescers, enzymes and chemiluminescent compounds.

Kinsky and his colleagues were the first to show that liposomes containing haptenated lipids could bind with an antibody and fix the complement thereof (Haxby et al., *Proc. Natl. Acad. Sci. USA,* 61 300 (1968); Alving et al., *Biochem.,* 8 1582 (1969); Kinsky et al., *Biochem.,* 8 4149 (1969)). The result was the lysis of the liposomes by the activated complement components.

Cole, U.S. Pat. No. 4,342,826, describes an immunoassay method which utilizes antigen-tagged, enzyme-encapsulated liposomes which are immunospecifically ruptured in the presence of the cognate antibody and an active complement. The assay utilizes the homogeneous phase reaction between the antibody and complement to release the enzyme marker. This complement mediated event has been the focal point for a large amount of literature (for a recent review, see Alving & Richards, *Liposomes,* Ostro, ed., 209–287 (Marcel Dekker, New York, 1983)).

Recently several noncomplement mediated liposome lytic assays have been developed. For example, binding of the antibody to haptens conjugated to a membrane lytic protein, melittin, blocks and liposome lytic activity of the melittin (Freytag et al., *Biophys. J.,* 45 360(a) (1984)). Binding of the antibody in the Lupus serum to liposomes containing cardiolipin prevents the lysis of the liposome by Mg^{+2} ions (Janoff et al., *Clin. Chem.,* 29 1587 (1983)). While no complement is required each of these assays requires either a membrane lytic molecule or ion.

Although the previously described assays may be quite sensitive, they often involve many steps, and are sometimes difficult to reproduce and/or automate. Thus, new and more efficient assays are desirable.

SUMMARY OF THE INVENTION

The present invention is directed to an immunoassay wherein the lysis of the liposomes is a direct consequence of the immune complex formation. The assay of this invention is as sensitive as RIA, providing rapid determinations, yet it does not require the presence of membrane lytic molecules, ions, or active complements.

This invention is directed to a new membrane lytic immunoassay. Accordingly, said membrane lytic immunoassay comprises the steps of:

(a) forming liposomes containing the analyte of interest and a marker compound;

(b) providing a solid phase inert support having attached thereto a receptor for the analyte of interest;

(c) mixing said test fluid with said receptor-solid phase support of step (b) for sufficient time to saturate said receptor with any analyte present in said test fluid;

(d) mixing said liposomes formed in step (a) with said saturated receptor-solid phase support from step (c);

(e) determining the presence of marker compound released by the liposomes in step (d).

In one embodiment of this assay, an antigen is first covalently coupled to a lipid and this antigen-lipid complex is used in conjunction with an otherwise non-bilayer forming lipid or mixture of lipids to form stable bilayer liposome vesicles which additionally contain a self-quenching fluorescent dye. When this antigen and dye containing liposome is brought into contact with an inert solid surface having attached thereto, antibody molecules, rapid binding occurs between the antigen-lipid complex and the antibody, disrupting the liposome and releasing the dye. Release of the dye can be quantified using standard fluorometric measurements. To assay the amount of antigen in a test sample, the sample, original or diluted, is first added to the inert solid surface to saturate the attached antibody. Thus, the subsequent liposome binding and dye release are reduced. The amount of antigen in an unknown sample is then determined by comparison with the amount in known standards. The invention is also directed to products useful in said assay, especially in kit form.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the stabilization of DOPE and DOPC liposomes with DNP-cap-PE. 90° light scattering of the sonicated lipid were measured for DOPE (a) and DOPC (b) liposomes;